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Bacterial Pollution Indicators in the Intestinal Tract of Freshwater Fish

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ABSTRACT

GELDREICH, EDWIN E. (Division of Water Supply and Pollution Control, Cincinnati, Ohio), AND NORMAN A. CLARKE. Bacterial pollution indicators in the intestinal tract of freshwater fish. Appl. Microbiol. **14**:429–437. 1966.—A study was made of the occurrence, distribution, and persistence of coliforms, fecal coliforms, and fecal streptococci in the intestinal tract of freshwater fish. A total of 132 fish representing 14 different species were used in various phases of these experiments. Examination of the intestinal contents of 78 fish from moderately polluted sections of the Little Miami River indicated that fecal coliform densities were lowest in bluegills (less than 20 per gram) and highest in catfish (1,090,000 per gram). Levels of fecal streptococci for these two species were 220 and 240,000 per gram, respectively. The occurrence of fecal coliforms in fish caught in this stream reflected the warm-blooded-animal-pollution level of the water. All fish used in this phase of the study were caught during July, August, and September when the water temperatures were between 13 and 18 C. The fate of fecal coliforms and *Streptococcus faecalis* in the fish intestine indicated that these organisms can probably survive and multiply when fish and water temperatures are 20 C or higher, but only when the organisms are retained in the gut for periods beyond 24 hr. Based on the biochemical reactions for 3,877 coliform strains isolated from 132 freshwater fish of 14 different species, 91.4% of all strains were composed of five IMViC types. In a similar study of the biochemical reactions of 850 streptococci isolated from the intestinal tract of 55 freshwater fish, the predominant strains included *S. faecalis* and various closely associated biotypes. No consistently recurring pattern for either coliforms or streptococci could be developed to identify species of fish investigated. The composition of the intestinal flora is, however, related in varying degree to the level of contamination of water and food in the environment.

In recent years, there has been considerable interest in studying the occurrence and distribution of the various bacterial pollution indicators in water and the associated environment. Reports of studies on potential natural pollution of water from warm-blooded animals, soils, vegetation, and insects have appeared in the literature. It has been suggested repeatedly that the bacterial flora of fish might reflect the bacteriological conditions of the water and thus be a potential indicator of pollution. This thesis assumes that coliforms, fecal coliforms, and fecal streptococci are not normal inhabitants of the gut of fish and have a short, unaltered survival in their intestinal tract. Few quantitative data can be found in the literature, however, on either the pollution that might be contributed by fish, or the degree of correlation between water quality and the number of bacterial pollution indicators in the fish intestine.

Organisms of the coliform and streptococcal groups have been isolated from the intestinal tract of various species of freshwater fish caught in relatively clean to moderately polluted waters in India (18), Norway (17), Canada (2, 3, 14), and the United States (12). Margolis (14) also reported that the bacterial flora of freshwater fish depends solely upon the fish's recent intake of food and the degree of contamination in the food and water. Gibbons (7, 8) reported similar results for fish caught from the marine environment, and both authors concluded that coliform bacteria are not usually associated with the normal intestinal flora of fish.

Although numerous microbiological studies have been made on fish intestinal material, many

of the data are qualitative and are based on technical procedures with limited sensitivity and selectivity. Insufficient data in the literature on quantitative recovery of coliforms, fecal coliforms, and fecal streptococci that could be applied to water pollution studies prompted this investigation. The research project was developed to include the following areas: (i) quantities of coliforms, fecal coliforms, and fecal streptococci in the intestinal contents of fish; (ii) possible correlation of these pollution indicators in fish with those in the water environment; (iii) survival and multiplication of fecal coliforms and streptococci in intestinal material; (iv) determination of any density differences and possible type differences of pollution indicators in the upper and lower intestine; (v) occurrence and percentage distribution of coliforms and streptococcal strains from the gut of a wide variety of fish; (vi) the effect of ingestion of specific tracer organisms and their retention in the fish intestine; and (vii) contribution of fish to pollution of a potable water.

MATERIALS AND METHODS

Bacteriological examinations were conducted as soon as possible after the fish were caught, except for fish caught in areas of Canada; Pymatuning Lake, Pa.; and Dale Hollow, Tenn., where the intestinal tracts were immediately removed aseptically and frozen for prompt shipment to this laboratory. Fish caught in the Ohio and Little Miami rivers and in several small Ohio lakes and those from experimental fish tanks were placed in plastic bags and examined immediately on return to this laboratory.

Most of the fish were taken with fish traps and large river nets set in the streams for 1 to 2 weeks during the summer; and a few were obtained by seining with a gill net, and the remainder were caught with a line. Fish used in the 250- and 500-gal tank experiments were taken with dip nets.

To determine the flora of the intestinal tract, the entire gut from stomach to anus was removed, and the contents were stripped out with sterile forceps. In many of the samples that contained large amounts of fecal material, a minimal amount of 0.5 g (wet weight) was obtained from both the upper and lower intestinal tract for use in split-sample studies. After they were weighed, the samples were transferred to appropriate amounts of sterile dilution water to make a 1:100 dilution. The material was homogenized in the dilution water by shaking with glass beads.

Fecal suspensions were examined for coliform bacteria by the "Confirmed" and "Completed" multiple-tube most probable number (MPN) procedures as described in *Standard Methods for the Examination of Water and Wastewater* (1). The fecal coliform test, as described by Geldreich et al. (4), consisted of confirming all positive presumptive tubes in EC broth at 44.5 ± 0.5 C. MPN for the fecal streptococcal group was determined by the "Tentative Test" (1) with azide dextrose (AD) presumptive broth, followed by confirmation of all positive tubes in ethyl violet azide (EVA) medium. All confirmed positive EVA tubes were verified by microscopic examination to be gram-positive cocci. Pour plates of KF streptococcus agar (13) were used for the isolation of colonies to be studied in subsequent species or group classification for the streptococcal types present. All quantitative data are reported as number of organisms per gram of sample.

Coliform strains used in the study of biochemical reactions were obtained through a primary isolation of colonies growing on the membrane filter. At least 20 to 50 isolated colonies with typical sheen were transferred to Phenol Red Lactose Broth (Difco) for fermentation tests and verified in brilliant green lactose broth. Streaking on E M B Agar (Difco) insured cultural purity. All purified strains were then inoculated with EC broth at 44.5 C and introduced into the media necessary for identification and classification according to IMViC types (1).

Possible survival and multiplication of a fecal coliform (++−− IMViC type) and *Streptococcus faecalis* var. *liquifaciens* in the intestinal material of fish were also investigated. To demonstrate growth within this material, intestinal contents of six bluegills and eight carp were prepared as a 1% suspension with triple-distilled water. These "media" were then chemically sterilized with ethylene oxide and heated to 60 C for 6 hr to drive off residual ethylene oxide. Flasks of these "media" were individually inoculated with pure cultures of the ++−− fecal coliform and *S. faecalis* var. *liquefaciens* and incubated at 10, 20, or 35 C. Plate counts on Brain Heart Infusion Agar (Difco) were made initially and after 24, 48, and 72 hr to obtain bacterial densities.

RESULTS AND DISCUSSION

Percentile distributions for the MPN values of the five-tube coliform "Completed Test" and for the fecal coliform and fecal streptococcal group tests on various species of fish and their river water environments are summarized in Table 1. Coliforms, fecal coliforms, and fecal streptococci were found in many of the 78 specimens of fish examined, including bluegills (*Lepomis macrochirus macrochirus*), bass (*Micropterus salmoides*), white crappies (*Pomoxis annularis*), gizzard shad (*Dorosoma cepedianum*), white suckers (*Catostomus commersonii commersonii*), northern redhorses (*Moxostoma aureolum*), carp (*Cyprinus carpio*), and channel catfish (*Ictalurus lacustris punctatus*) caught in moderately polluted sections of the Little Miami River. Fecal-coliform densities were lowest in bluegills; in 75% of the samples examined, the value was below the minimal level that could be detected in the amount of fecal material available. The 330 coliform and 220 fecal streptococci values at the 75th percentile level reflect the low densities of these indicators also in bluegill fecal material. Catfish contained

TABLE 1. *Percentile distribution of bacteria by quartile of MPN values per gram of fish intestinal material**

Fish species	No. of specimens	Test								
		Completed coliform			Fecal coliform			Fecal streptococci		
		25%	50%	75%	25%	50%	75%	25%	50%	75%
Sunfish										
Bluegill	6	<20	20	330	<20	<20	<20	<20	<20	220
Bass	13	81	490	221,000	<20	330	23,000	23	60	79,000
White crappie	9	<20	3,300	490,000	<20	330	7,900	49	240	23,000
Herring										
Gizzard shad	11	33	490	79,000	8	70	46,000	13	230	10,900
Suckers										
White sucker	6	490	630	7,000	50	70	490	79	79	940,000
Northern redhorse	6	13,000	17,200	172,000	2,300	4,600	13,000	42,600	130,000	790,000
Minnows										
Carp	5	17,200	221,000	278,000	1,300	7,900	7,900	3,300	3,300	6,300
Catfish										
Channel catfish	22	84,000	221,000	7,900,000	3,300	49,000	1,090,000	3,300	130,000	240,000
Little Miami River†	13	141	1,700	2,200	55	300	370	22	40	100

* All fish were caught in the Little Miami River during July, August, and September.
† Densities per 100 ml of water.

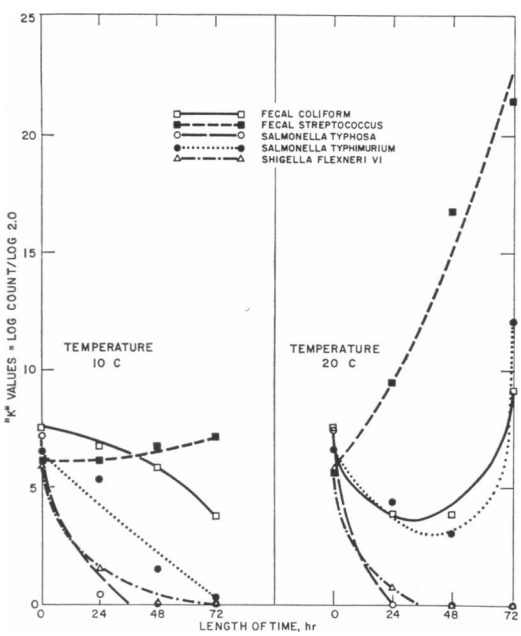

FIG. 1. *Persistence of selected enteric bacteria in carp intestinal material.*

the largest bacterial concentration, with upper quartile values of 7,900,000 coliforms, 1,090,000 fecal coliforms, and 240,000 fecal streptococci per gram of feces. All fish used in these experiments were caught during July, August, and September when the water temperature was between 13 and 18 C.

Examination of water sampled at the various fish-trap locations indicated lower densities for the pollution indicators in river water than in the fish. Upper quartile values for total coliforms, fecal coliforms, and fecal streptococci were 2,200, 370, and 100 per 100 ml, respectively. These data suggest that the major source of the pollution indicators in fish from the Little Miami River was probably related to the intake and degree of contamination of food ingested. Margolis (14) reported the same observation in a study of the effect of fasting on bullheads and speckled trout in tank experiments. Our tank experiments with carp and bluegills indicated a higher recovery for the pollution indicators in fish guts when the water temperatures ranged from 16 to 21 C than when they ranged from 1 to 10 C. In the latter case, fish were not actively feeding and intestinal tracts were mostly empty.

Another possible reason for the higher counts in fish than in their water environment could be a result of bacterial multiplication within the fish gut. Persistence studies for a fecal coliform, a fecal streptococcus, *Salmonella typhosa*, *S. typhimurium*, and *Shigella flexneri* in previously sterilized fish fecal material are demonstrated in Fig. 1 and 2. Subjecting this material to ethylene oxide sterilization does eliminate any potential composite effect of interaction and interrelationships between the original microbial flora and the persistence of experimental organisms. The influence, however, of such environmental factors as *p*H of fecal material, chemical toxicity of waste

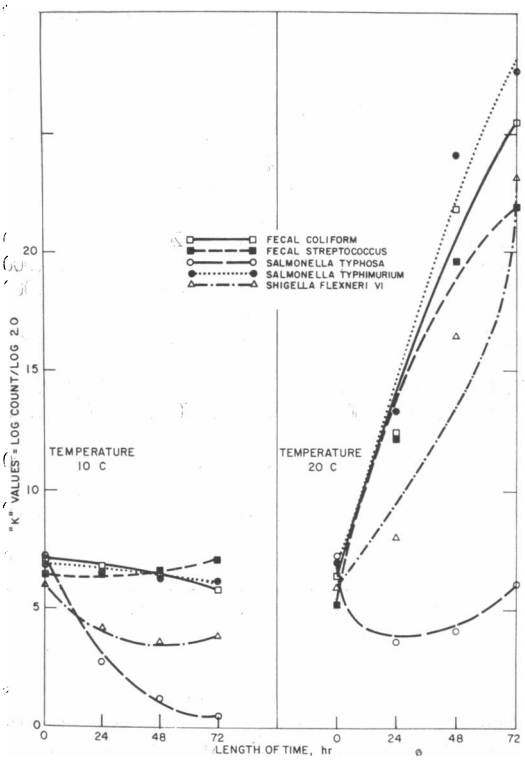

FIG. 2. *Persistence of selected enteric bacteria in bluegill intestinal material.*

products, and temperature variations can be observed in growth studies of sterilized fecal discharges.

The data presented in Fig. 1 were obtained in studies of fecal material from carp caught in several locations, whereas data for Fig. 2 were obtained in similar studies of bluegill specimens. The curves are based on average values obtained from 3 to 11 replicate experiments for each bacterial strain and fish species. These data indicate a progressive die-away for the fecal coliform and three pathogenic strains of bacteria at 10 C, with only a slight increase of approximately two generations in 72 hr for fecal streptococci in fecal material from both carp and bluegill. Since body temperatures of fish are essentially the same as the temperature of the water they inhabit, data from the 10 C experiments suggest that no significant multiplication of the pollution organisms occur in the gut of fish over a 3-day period during winter and early spring.

When the experiments with both species were repeated at 20 C (summer water temperature), a pronounced increase in growth of fecal streptococci occurred within 48 hr. The bluegill samples had, in addition, a similar increase in bacterial density for the fecal coliform, *S. typhimurium*, and *S. flexneri*. With 72-hr incubation, some growth occurred for the fecal coliform and *S. typhimurium* in carp fecal material, but not of the magnitude found in bluegills. In fecal material from bluegills, *S. flexneri* significantly increased with 48-hr incubation, but was completely killed in carp material for the same time interval. These bacterial growth differences in the two species of fish may be related to food source, waste products, and *p*H of the intestinal material. The *p*H of carp fecal material averaged 7.3, whereas that of the bluegill was 8.0. The magnitude of growth of these organisms in living fish could be altered by the presence of a mixed microbial population containing phages or antagonistic substances of pseudomonads. Havens and Dehler (11) found evidence that the presence of *Pseudomonas aeruginosa* in the intestinal flora of the top minnow *Gambusia affinis* inhibited the growth of *Escherichia coli*. The blue-green alga *Schizothrix*

TABLE 2. *Bacterial densities (per gram) in upper- and lower-intestinal contents of fish*

Fish species	Water temp	Total coliforms		Fecal coliforms		Fecal streptococci	
		Upper	Lower	Upper	Lower	Upper	Lower
Bluegill	Summer (16–20 C)	26	172	<2	<2	<2	<2
River quillback		490	790	9	70	79	79
Northern redhorse		130	2,120,000	33	7,900	12	17,200
White sucker		630	33,000	79	10,900	21	7,900
Carp		17	3,300	5	490	<2	<2
Catfish		7,900	84,000	1,720	3,300	1,300	3,300
Carp A	Winter (1–10 C)	11	17	<2	<2	130	4
Carp B		2	<2	<2	<2	23	70
Carp C		34	39	<2	<2	<2	<2
Carp D		2,210	17,700	<2	2	<2	33

TABLE 3. *Occurrence of coliform types in fecal material from freshwater fish*

IMViC type	Sunfish*		Gizzard shad		Sucker†		Carp		Catfish		Summary	
	No. of strains examined	Per cent isolated	No. of strains examined	Per cent isolated	No. of strains examined	Per cent isolated	No. of strains examined	Per cent isolated	No. of strains examined	Per cent isolated	No of strains examined	Per cent isolated
++−−	185	18.9	92	41.4	131	34.3	73	9.1	572	38.3	1,053	27.2
−−++	49	5.0	47	21.2	25	6.5	56	7.0	206	13.8	383	9.9
−+−−	16	1.6	2	0.9	7	1.8	13	1.6	38	2.5	76	2.0
+++−	0	0.0	3	1.4	3	0.8	14	1.8	7	0.5	27	0.7
−+−+	434	44.4	31	14.0	156	40.8	289	36.1	360	24.1	1,270	32.8
++−+	246	25.1	4	1.8	11	2.9	152	19.0	41	2.7	454	11.7
−+++	19	1.9	6	2.7	6	1.6	66	8.3	40	2.7	137	3.5
++++	24	2.5	22	9.9	36	9.4	112	14.0	187	12.5	381	9.8
+−++	5	0.5	2	0.9	2	0.5	13	1.6	36	2.4	58	1.5
−−−+	0	0.0	0	0.0	2	0.5	1	0.1	6	0.4	9	0.2
−++−	0	0.0	5	2.3	1	0.3	9	1.1	2	0.1	17	0.4
−−+−	0	0.0	2	0.9	1	0.3	1	0.1	0	0.0	4	0.1
+−+−	0	0.0	6	2.7	1	0.3	0	0.0	0	0.0	7	0.2
+−−+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
+−−−	0	0.0	0	0.0	0	0.0	1	0.1	0	0.0	1	0.0
Total	978	—	222	—	382	—	800	—	1,495	—	3,877	—
EC-positive	182	18.6	103	46.4	154	40.3	65	8.1	720	48.2	1,224	31.6

* Sunfish include bluegill, bass, and white crappie.
† Suckers include white sucker and northern redhorse.

calcicola, which developed a dense "algal bloom" in one of our outdoor experimental tanks, was also demonstrated by laboratory experiments to be toxic to a fecal coliform strain within 24 hr. If fish had ingested large amounts of this alga, it is assumed that a similar toxic reaction might have occurred in their intestinal tract.

Although these factors might limit survival or growth of pollution indicators and pathogens in the fish intestinal tract, there is also evidence that growth might occur if conditions of temperature and retention of feces in the intestine are favorable. Retention of fecal excreta is related to gut motility, and data on bacterial densities for the three pollution indicators in the upper and lower intestine (Table 2) suggest such retention. Bacterial counts in examples of six fish species indicate a definite trend toward higher densities for total coliform, fecal coliform, and fecal streptococci in material from the intestinal sections adjacent to the anus. Since fish do not have an indigenous bacterial flora (10, 14, 15, 19), these pollution organisms must have been introduced into the fish by contaminated food and water. The resulting increases in densities in the lower gut may be related to slow gut motility, which, coupled with warm summer water temperatures, would permit some bacterial multiplication prior to excretion. Table 2 shows a low order of increase in densities for intestinal material from the upper and lower gut of carp in water at 1 to 10 C. No evidence of toxicity to *E. coli* or *S. faecalis* that might be associated with bile or low *p*H in the upper intestinal material could be found in various split samples.

The biochemical reactions of 3,877 coliform strains isolated from 132 freshwater fish of 14 different species are reported in Table 3. These data are divided into fish groups or individual species with similar eating habits. Only the IMViC −+−+ and ++−− types reached percentages that might be considered predominant. The −+−+ coliform type occurred most frequently in sunfish, suckers, and carp, and the ++−− type was most common to gizzard shad and catfish. Seaburg and Moyle (16) studied feeding habits of freshwater fish and found that bluegills, pumpkinseeds, and crappies feed mostly on insects in the early summer. Previous studies of soil and insects (5, 6) indicated that the −+−+ coliform type occurred most frequently, i.e., 48.1% in soil and 30.6% from insects. Since sunfish feed on insects, the similarity of percentages for −+−+ type in those fish and insects is interesting, as well as the percentage of fecal coliforms in sunfish (18.6%) and insects (14.9%). Data on coliform types from all fish indicate that 91.4% of all strains were composed of five IMViC types. The remaining coliform types occurred in varying amounts of less than 5%.

The occurrence of fecal coliforms in fish could be a reflection of the pollution level of their

TABLE 4. *Occurrence of coliform types in fish and their water environment*

IMViC type	Little Miami River				Fish hatchery tanks			
	Fish*		Water		Fish†		Tanks	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
++−−	263	27.1	40	25.8	28	2.7	1	0.5
−−++	128	13.2	35	22.6	8	0.8	0	0.0
−+−−	24	2.5	3	1.9	0	0.0	0	0.0
+++−	7	0.7	2	1.3	17	1.6	0	0.0
−+−+	333	34.4	36	23.2	499	48.0	124	62.0
++−+	47	4.9	10	6.5	320	30.8	50	25.0
−+++	22	2.3	7	4.5	26	2.5	1	0.5
++++	118	12.2	12	7.7	129	12.4	24	12.0
+−++	10	1.0	8	5.2	6	0.6	0	0.0
−−−+	2	0.2	0	0.0	1	0.1	0	0.0
−++−	7	0.7	1	0.7	5	0.5	0	0.0
−−+−	2	0.2	0	0.0	0	0.0	0	0.0
+−+−	6	0.6	0	0.0	0	0.0	0	0.0
+−−+	0	0.0	1	0.7	0	0.0	0	0.0
+−−−	0	0.0	0	0.0	0	0.0	0	0.0
Total	969	—	155	—	1,039	—	200	—
EC-positive	298	30.8	48	31.0	11	1.1	6	3.0

* Data from eight species of fish.
† Data from carp and bluegills.

water environment in addition to their feeding habits. Table 4 shows the fecal coliform percentage, based on EC-positive strains, to be 30.8% for fish, which is essentially identical with that for water (31.0%) from the same location in the Little Miami River. Analysis of the coliform types from Little Miami River fish indicated that 86.9% of all types were represented by ++−−, −−++, −+−+, and ++++. These same four types were represented in 79.3% of all coliforms isolated from fish collected at this location.

Experiments with bluegills and carp kept in 250- and 500-gal tanks at the Newtown Fish Hatchery also support the thesis that the coliform types present in fish are the same as those in their water environment. The fish food contained less than two fecal coliforms per gram, and the tank water yielded 3.0% fecal coliforms among the 200 strains examined. This value was essentially the same as the 1.1% fecal coliforms among the 1,039 coliform strains isolated from bluegills and carp in the tanks. Three coliform types (−+−+, ++−+, and ++++) represented 91.2% of the strains from carp and bluegills used in these tank experiments, whereas the same three coliform types were represented in 99.0% of the strains isolated from the tank water.

The results of the biochemical tests with 850 streptococci isolated from the fecal material of 55 freshwater fish are given in Table 5. The species of fish represented included bluegill, bass, white crappie, gizzard shad, white sucker, carp sucker, northern redhorse, carp, catfish, and northern pike. A preliminary analysis of data from different species of fish or fish of similar feeding habits did not reveal any significant differences; therefore, the data are presented in a summary table.

The predominant streptococci grew at both 45 and 10 C and were found in 88.5%, or 752, of the total 850 strains. These included strains of *S. faecalis* and various closely associated biotypes. Only 58 strains, or 6.8%, of the streptococci were capable of growth at 45 and not at 10 C. Such a grouping would include one or more of the following streptococcal strains: *S. bovis*, *S. equinus*, and *S. salivarius*. Finally, only 40 strains were capable of growth in Brain Heart Infusion broth at 10 C, but not at 45 C; these included strains of *S. lactis*, among others.

A previous study of the streptococci isolated from insects (6) demonstrated that peptonization of milk appears to be a relatively common characteristic of fecal streptococci isolated from insects. In that investigation, 45.6% of all streptococcal strains from insects were positive. Because some fish feed on large quantities of insects, the data were analyzed for any significant percentage of litmus milk peptonization. No positive strains were found in fecal material from pike. Seaburg and Moyle (16) examined the

stomach contents of 203 northern pike and found fish to be the major food item in all but one, which consisted only of frogs. Although gizzard shad are normally considered to feed primarily on plankton, Price (*unpublished data*) found that gizzard shad may on occasion feed heavily on insects. Isolation of streptococcal strains in this study of gizzard shad showed 59.6%, or 28 strains, were positive for litmus milk peptonization, which indicated that they possibly were derived from insects.

To summarize all the streptococcal data on milk peptonization in fish, 137 strains, or 16.1% of the 850 cultures, were positive. In our study of 3,158 streptococcal strains from feces of warm-blooded animals, we found a similar distribution of organisms with this characteristic, i.e., 16.9%, or 533 positive cultures. Thus, the data presented here indicate that streptococci which peptonize litmus milk are not permanent residents of the fish gut and, when present, may be related to the intake of insects as food.

An experiment was conducted in which carp and bluegills were exposed to fecal coliform and fecal streptococci tracer strains that were added to tank water, but not to the food. The fecal coliform strain was capable of H_2S production at 44.5 C, and the fecal streptococci peptonized litmus milk in 18 to 24 hr. These two organisms were not detected in the tank water, fish food, or fish prior to experimental dosage. The tank was dosed with saline-washed cells of 24-hr-old cultures of the two tracer organisms. Then, after various exposure periods, fish were sacrificed for evidence of ingestion and retention of these organisms in their intestinal tract.

Data in Table 6 demonstrate that tracer organisms ingested by bluegills and carp were retained in the intestinal tract for 9 to 14 days. Data on this experiment, and other data not included in this table, show that the contamination of the water environment must remain at high levels if continued recovery of these organisms from fish guts is to be expected. Similar conclusions for *E. coli* serotypes in trout were reported by Glantz and Krantz (9). They detected *E. coli* serotypes for periods of 1 to 14 days in trcut that received food and water dosed with *E. coli* prior to retention studies.

Once fish have acquired pollution or pathogenic organisms, or both, in their gut, it is conceivable that they could become carriers of such bacteria to clean stream areas some distance from a polluted water environment. Results of tank experiments designed to study contamination of a potable spring water by fish exposd to high levels of coliforms and fecal streptococci are reported in Table 7. At the time of transfer of three carp and three bluegills, the tank contained approximately 250 gal of spring water and was

TABLE 5. *Biochemical reactions of 850 streptococci isolated from fecal material of 55 freshwater fish**

No. of strains	Growth at		Growth in		Reduction of		Final pH in 1% glucose	Hydrolysis of starch	No. of strains peptonizing milk	Arginine hydrolysis
	10 C	45 C	1.5% NaCl	Broth (pH 9.6)	Methylene blue (0.1%)	Potassium tellurite 1:2,560				
535	+	+	+	+	+	+	4.0–4.7	0	119	+
16	+	+	+	+	0	+	4.1–4.4	0	9	+
5	0	+	+	+	0	+	4.9–6.5	0	0	+
43	+	+	+	0	+	+	4.1–4.4	0	0	+
3	0	+	+	0	+	+	4.8–6.4	0	0	+
48	0	+	+	0	0	+	4.8–6.5	0	0	+
33	+	+	+	+	0	+	4.3–5.2	0	0	0
29	+	+	+	+	+	+	4.3–4.4	0	6	0
1	+	+	+	+	+	0	4.5	0	0	0
1	+	+	0	+	+	0	4.2	0	0	0
16	+	+	0	+	+	+	4.2–4.4	0	0	+
32	+	+	0	+	0	+	4.2–4.4	0	0	+
2	0	+	0	+	0	+	4.2–4.4	0	0	+
22	+	+	0	+	0	+	4.4	0	0	0
1	+	+	0	+	+	+	4.4	0	0	0
1	+	+	0	0	0	+	4.3	0	0	+
1	+	+	0	0	0	0	4.3	0	0	+
14	+	0	+	+	+	+	4.2–4.4	0	0	+
26	+	0	0	+	±	+	4.2–4.5	0	0	+
21	+	+	+	+	+	+	4.2–4.7	+	3	+

* All strains were catalase-negative; all grew in 40% bile broth.

TABLE 6. *Experimental exposure of carp and bluegill to fecal coliforms and fecal streptococci tracer strains added to tank water**

Tank water† (counts per 100 ml)				Fish guts (counts per gram)		
Time after dosage	*p*H	Fecal coliform	Fecal streptococci	Species	Fecal coliform	Fecal streptococci
1 hr	7.1	79,000	7,000,000	—	—	—
1 day	7.1	13,000	141,000	—	—	—
7 days	7.1	27	460	Carp	<2	9
				Bluegill	490	1,720
9 days	6.9	5	130	Carp	11	4,600
				Bluegill	<2	26
14 days	6.5	<2	23	Carp	23	230
				Bluegill	<2	<2
16 days	6.8	<2	23	Bluegill	<2	<2

* No fecal coliform or fecal streptococci detected in fish, water, or food prior to dosing.
† Tank water temperatures ranged from 16 to 18 C.

TABLE 7. *Contribution of polluted fish to pollution of potable spring water[a] in tank experiments*

Tank water[b] (MPN counts per 100 ml)					Fish guts (MPN counts per gram)			
Time after fish added (days)	*p*H	Total coliform[c]	Fecal coliform	Fecal streptococci	Species	Total coliform[c]	Fecal coliform	Fecal streptococci
2	7.2	49,000	<2	49	Carp	330,000	<2	23
					Bluegill	172	<2	<2
7	7.3	3,300	<2	49	Carp	70,000	<2	1,090
					Bluegill	<20	<20	<20
9	7.2	27,800	<2	1,410	Carp	24,000,000	<2	175,000
					Bluegill	34,800,000	22	10,900,000
5[d]	7.5	1,300	<2	2				
12[d]	7.3	141	<2	17				

[a] Less than two total coliforms, fecal coliforms, and fecal streptococci per 100 ml of spring water.
[b] Tank water temperatures ranged from 16 to 20 C.
[c] Completed test for total coliform densities.
[d] Time after fish were removed from the tank.

aerated to maintain sufficient dissolved oxygen to support the fish life. The results indicated that the contamination of the potable spring water was caused by organisms associated with fish intestinal wastes and slimes from body surfaces. Similar experiments were also performed at tank temperatures of 1 to 5 C and 7 to 10 C, with essentially the same results.

The data presented constitute strong evidence that there is no permanent coliform or streptococcal flora in the intestinal tract of fish. The composition of the intestinal flora is related in varying degrees to the level of contamination of water and food in the environment. These flora can be modified by many factors, such as feeding activity, available food, and degree of water pollution. They can also be altered by antibiotic effects of ingested algae, *Pseudomonas* species, or other bacteriological agents in the fish gut. Fish may also be carriers of pollution from warm-blooded animals for periods up to approximately 7 days, and could in this manner transfer potential pathogens to clean water areas.

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